Association between abnormal microbiological flora of the lower genital tract in early pregnancy and socio-economic, demographic and environmental risk factors

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key words: bacterial vaginosis, risk factors, bacterial infection, pregnancy

SUMMARY

Background: The main aim of this study was to determine the socioeconomic, demographic and environmental factors which may be associated with the occurrence of pathological microflora of the lower genital tract in early pregnancy.

Material and methods: A group of 96 pregnant women was selected at random from the patients of 10 district maternity units in the Łódź region of Poland. Only singleton pregnancies below 24 weeks were qualified for inclusion in the survey. A standard questionnaire covering medical, socio-economic, demographic, constitutional, and environmental items was administered to every subject and checked against medical records. Based on microbiological results, two groups of pregnant women were distinguished: Group I, with normal cervicovaginal flora, predominantly Lactobacillus spp. with coagulase-negative staphylococci and viridans streptococci, and Group II, with abnormal flora. The latter included two subgroups: IIA, intermediate microbial flora, dominated by M. hominis, U. urealyticum, G. vaginalis, gram-negative anaerobic rods, Ch. trachomatis, and few Lactobacillus spp, and IIB, highly abnormal flora, containing similar microbial components as in IIB but without Lactobacillus spp.

Results: Based on the results of microbiological culturing, 18 (18.7%) of the 96 women examined were classified to Group I, and 78 (81.2%) to Group II: 32 (33.3%) in group IIA and 46 (47.9%) in IIB. Groups IIA and IIB were combined for further analysis. An excessive risk of abnormal vaginal flora was observed in connection with such socio-economic factors as marital status, unemployment, and smoking, Moreover, the first pregnancy was also found to be a potential risk factor for this pathology. The risk of developing abnormal vaginal flora, although exceeding unity for each of these factors, was not considered statistically significant.

Conclusions: Socio-economic and environmental factors may influence the course and outcome of pregnancy. Pregnant women who present with risk factors for abnormal cervicovaginal microflora should be included in comprehensive prenatal surveillance, which enables early detection and treatment of this pathology.

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BACKGROUND

Abnormal microbiological flora of the lower genital tract during pregnancy, cervicovaginal infections and bacterial vaginosis (BV), are familiar causes of perinatal complications [1]. Microorganisms ascending from the lower genital tract produce local inflammation, sub-clinical chorioamnionitis leading to preterm rupture of membranes (PROM) and/or preterm labor, and possibly preterm birth [2–7]. According to Goldenberg et al. [8], BV accounts for 40% of the attributable risk for spontaneous birth at less than 32 weeks of pregnancy.

Bacterial vaginosis is a clinically definable condition that is frequently diagnosed in the primary care setting. Changes in the nomenclature, in views on causative microorganisms, and in treatment have caused confusion over the last century. BV is currently characterized as a change from the normal vaginal ecosystem to a reduced concentration of normally existing aerobic bacteria and Lactobacillus species, and increased concentration of anaerobic bacteria, such as Gardnerella vaginalis, Mobiluncus, Bacteroides, Prevotella and Mycoplasma species [9,10]. However, more than half of the clinically diagnosed patients are asymptomatic [11]. The microorganisms occurring and their relative concentrations vary among pregnant women with BV [12].

Given the growing evidence for a relationship between abnormal microbiological flora of the lower genital tract and the risk of preterm delivery, it would be a matter of considerable interest to investigate the possible role played by certain socio-economic characteristics of pregnant women, such as educational level, employment, marital status and economic condition, in the development of this pathology. Such research may also be helpful in clarifying the mechanism through which socio-economic factors contribute to an increased risk of preterm delivery [13,14]. Should any evidence be found that socially underprivileged women do in fact have a higher prevalence of abnormal cervicovaginal flora during pregnancy, this could be postulated as an important link between the socio-economic and demographic factors on the one hand, and preterm delivery on the other.

The primary purpose of our research was to determine the socio-economic, demographic and environmental factors that may be associated with the occurrence of pathological microflora of the lower genital tract in early pregnancy.

MATERIAL AND METHODS

Study population

A group of 96 pregnant women was selected at random from the patients of 10 district maternity units in the Lodz region of Poland. Only singleton pregnancies below 24 weeks were qualified for inclusion in the survey. A standard questionnaire covering medical, socio-economic, demographic, constitutional, and environmental aspects was given to every subject and checked against medical records.

Bacteriological examination

For the qualitative and quantitative assessment of biocenosis in the lower genital tract, vaginal and cervical swabs were collected from the pregnant women under study. First, bacteriological tests of cervical swabs were made to screen for Ch. trachomatis, M. hominis and U. urealyticum. The Ch. trachomatis antigen was detected by direct immunofluorescence assay (BioMerieux). Commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used for the isolation, identification, and differential titration of genital mycoplasmas. Identification was based on the specific hydrolysis of urea (U. urealyticum) or arginine (M. hominis) by the species present in the specimen, which is indicated by a change in the color of the well containing the relevant substrate, without clouding in the medium. Titration based on dilution in liquid medium is expressed as the number of cfu per ml of specimen. This technique allows for titration at the level of ca. 10³ cfu/ml and 10⁴ cfu/ml, the accepted threshold levels of pathogenicity [15].

The vaginal swabs were tested for other aerobic and anaerobic bacteria. The swabs were placed in 3 ml pre-reduced sterile saline. 1:10 serial dilutions from 10⁻¹ to 10⁻⁸ were prepared. Each of the dilutions made from swabs was inoculated onto appropriate plates [16].

Sheep blood agar, MacConkey, D-Coccoesel agar, Gardnerella agar, Azide blood agar (Bio-Merieux) and Staphylococcus Medium 110 (Oxoid Ltd) plates were used to isolate aerobic organisms. Schaedler blood agar (BioMerieux) and Rogosa agar (Oxoid Ltd) were inoculated for anaerobic cultures. After the incubation period, the anaerobic and aerobic bacteria were identified by biochemical tests, API (BioMerieux).
Based on the microbiological results, two groups of pregnant women were distinguished:

- **Group I**, with normal vaginal flora, predominantly *Lactobacillus spp.* with coagulase-negative staphylococci and viridans streptococci;

- **Group II**, with abnormal flora.

**Group II** included two subgroups:

- **IIA**, intermediate microbial flora, with predominant *M. hominis*, *U. urealyticum*, *G. vaginalis*, gram-negative anaerobic rods, *Ch. trachomatis* and few *Lactobacillus spp*;

- **IIB**, highly abnormal flora, containing similar microbial components as in IIA, but no *Lactobacillus spp.* [17].

### Statistical analysis

Odds ratios (OR) were calculated to evaluate risk factors. Statistical analysis was conducted using EPI INFO software, taking into account the odds ratios and 95% confidence intervals (CI).

### RESULTS

#### Microbiological characteristics

The average pregnancy duration was 19 weeks, and the average age of the subjects was 26.1. Based on the results of microbiological culturing, 18 (18.7%) of the 96 women examined were classified to Group I, and 78 (81.2%) to Group II: 32 (33.3%) in group IIA and 46 (47.9%) in group IIB.

Among the facultative microflora, *G. vaginalis* was more frequent among women from Group IIB than IIA, although the differences were not significant. The mean concentration of these bacteria increased from $10^4$ cfu/ml in the IIA subjects to $10^5$ cfu/ml in the subjects from Group IIB. Likewise, genital mycoplasmas, e.g. *U. urealyticum* ($10^4$ cfu/ml), were more frequent among subjects from IIB than IIA. *Ch. trachomatis* was detected only in 10 (31.2%) subjects from IIB. Anaerobic gram-negative rods were the most frequently isolated of all anaerobic microorganisms. No significant differences with respect to the occurrence of *Prevotella* spp. and *Fusobacterium* spp. were found in the subgroups of Group II.

#### Socio-economic and environmental factors

Only minor differences occurred between the two subgroups of Group II in the indices of the microbial species examined; accordingly, in further data analysis Group II is treated as a whole, without respect to subgroups.

The proportion of women with a low educational level was 37.1% in Group II, compared with 27.8% in Group I (Table 1). The risk of developing abnormal vaginal flora, although exceeding unity, did not reach the level of statistical significance. The mean number of these bacteria increased from $10^4$ cfu/ml in the IIA subjects to $10^5$ cfu/ml in the subjects from Group IIB. Likewise, genital mycoplasmas, e.g. *U. urealyticum* ($10^4$ cfu/ml), were more frequent among subjects from IIB than IIA. *Ch. trachomatis* was detected only in 10 (31.2%) subjects from IIB. Anaerobic gram-negative rods were the most frequently isolated of all anaerobic microorganisms. No significant differences with respect to the occurrence of *Prevotella* spp. and *Fusobacterium* spp. were found in the subgroups of Group II.

### Table 1. The relative risk of abnormal microbiological flora of the lower genital tract and the socio-economic, demographic and environmental risk factors.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Physiological microbiological flora (n=18)</th>
<th>Abnormal flora (n=78)</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>3</td>
<td>16.7</td>
<td>8</td>
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<tr>
<td>21-30</td>
<td>9</td>
<td>50.0</td>
<td>59</td>
</tr>
<tr>
<td>&gt;30</td>
<td>6</td>
<td>33.3</td>
<td>11</td>
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<tr>
<td><strong>Education</strong></td>
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</tr>
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<td>Primary</td>
<td>5</td>
<td>27.8</td>
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<tr>
<td>College</td>
<td>13</td>
<td>72.2</td>
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<td><strong>Marital Status</strong></td>
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<tr>
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<td>14</td>
<td>77.8</td>
<td>57</td>
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<tr>
<td>Unmarried</td>
<td>4</td>
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<td>21</td>
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<td><strong>Employment</strong></td>
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<tr>
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<td>32</td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
<td>66.7</td>
<td>46</td>
</tr>
<tr>
<td><strong>Economic situation</strong></td>
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<tr>
<td>Poor</td>
<td>3</td>
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<td>13</td>
</tr>
<tr>
<td>Average good</td>
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<td>83.3</td>
<td>65</td>
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<td><strong>Smoking</strong></td>
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<tr>
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<td>14</td>
<td>77.7</td>
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<tr>
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<td>22.3</td>
<td>22</td>
</tr>
<tr>
<td><strong>Previous pregnancies</strong></td>
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<td></td>
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<tr>
<td>0</td>
<td>7</td>
<td>38.9</td>
<td>41</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
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<td>25</td>
</tr>
<tr>
<td>&gt;1</td>
<td>4</td>
<td>22.2</td>
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<td><strong>Previous spontaneous abortions</strong></td>
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<tr>
<td>Yes</td>
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</tr>
<tr>
<td>No</td>
<td>14</td>
<td>77.8</td>
<td>62</td>
</tr>
</tbody>
</table>

Based on the microbiological results, two groups of pregnant women were distinguished:

- **Group I**, with normal vaginal flora, predominantly *Lactobacillus spp.* with coagulase-negative staphylococci and viridans streptococci;

- **Group II**, with abnormal flora.
a potential risk factor for abnormal vaginal flora (OR=1.64 (0.45–3.77)) since the percentage of primipara women in Group I was 38.9%, compared to 52.6% in Group II. No such tendency was observed in analyzing the effect of the subject’s age or economic status.

**DISCUSSION**

In clinical practice bacterial vaginosis is usually diagnosed if at least three of the four composite criteria described by Amsel et al. [18] are satisfied. This is subject to inter-observer error, since most of the criteria are subjective. The use of the term ‘abnormal microbiological flora’ for purposes of the present analysis, in place of bacterial vaginosis or single pathogens, enables better classification of subjects into particular pathological groups, and may also shed some light on the observed differences among studies. Several investigators have shown that BV is associated with more frequent occurrence of various bacteria, including *G. vaginalis, M. hominis, U. urealyticum*, and anaerobic gram-negative rods (especially *Prevotella, Fusobacterium, Bacteroides Eubacterium* species) [21,22]. It has also been documented that vaginal infections (whether or not associated with bacterial vaginosis) may be of importance in many perinatal complications [23–29]. *Ureaplasma urealyticum* and *Mycoplasma hominis*, two genital mycoplasmas, are the most common organisms isolated in the perinatal period that either cause or are associated with poor perinatal outcomes [30–34]. Since these microbes are capable of TNF-alpha induction and stimulating nitric oxide synthase from murine macrophages, they may induce perinatal disease by producing proinflammatory mediators [35]. The mediators interact with inflammatory cells and either induce, act as a catalyst, or augment inflammation, which in turn leads to a poor pregnancy outcome.

Two groups of researchers have reported the results of longitudinal studies of bacterial vaginosis in pregnancy. Both centers found that intermediate flora are the most often observed in pregnancy, often reverting to bacterial vaginosis. The Vaginal Infections and Prematurity Study Group (USA) reported that bacterial vaginosis persisted throughout pregnancy in 88% of women in whom it was present at the 23rd to 26th week of gestation. The flora reverted to normal in approximately one-third of the women who had intermediate flora initially, and developed into BV in another one-third [36]. Hay et al. [37] found that women rarely develop BV as pregnancy progresses, and if initially present it remits in approximately half of the women reaching term. 76% of the women who had BV at the 28th week of gestation continued to have it at 36 weeks. They also showed that BV detected early in the second trimester of pregnancy is strongly associated with later miscarriage and preterm birth. Therefore, women should be screened and treated for BV no later than in the early second trimester of pregnancy.

We have no data on whether the presence of BV at conception or very early in pregnancy is associated with loss of pregnancy in the first trimester. Nor do we know the gestational age at which bacteria can ascend into the uterine cavity. The finding that chorioamnionitis is usually concentrated around the internal os of the cervix suggests that the microorganisms which ascend during pregnancy may be of some significance here [38].

We postulated that there might be an association between abnormal cervicovaginal flora and the socio-economic, demographic or/and environmental factors which are known to be the most essential risk factors for pregnancy outcome. Low educational level, single marital status, unemployment, primiparity and cigarette smoking constitute significant risk factors for abnormal microbial flora in early pregnancy. In a cross-sectional study, Wessel et al. [39] observed that bacterial infection among pregnant women was related to young age and single marital status. In another study, the woman’s age, marital status, number of pregnancies, smoking, and alcohol or drug abuse were not associated with the development of infection. Only such factors as African race and older gestational age at the first prenatal visit constituted significant risk factors [40]. This study was conducted among adolescents younger than 19 years of age, and only chlamydial infection was evaluated, which may be the explanation for the differences observed. In a Spanish population, also, no association was found between race, parity, education, marital sta-
tus, smoking, and the presence of BV during pregnancy [41].

Smoking during pregnancy constitutes a risk factor for abnormal microbiological flora of the lower genital tract and preterm delivery. Cnattingius et al. [5] underline that primiparous smokers are at an especially high risk for low birth weight and preterm delivery. However, the mechanism through which these factors can negatively affect pregnancy outcome remains unclear. A possible solution is suggested in the study conducted by Pavlova and Tao [42]: it may be that smoking reduces the number of vaginal lactobacilli. The chemicals contained in cigarette smoke have been analyzed in vitro to determine their role in reducing lactobacilli. The results showed that even trace amounts of benzo(a)pyrene diol epoxide (BPDE), found in the vaginal secretion of smoking women, significantly increased phage induction in vaginal lactobacilli, thus reducing their number and, consequently, increasing the overgrowth of anaerobic bacteria. Pastore et al. observed that fetal fibronectin was associated positively with BV, but only among women who smoke [43]. These data were not confirmed by Goldenberg et al. [44].

There are race/ethnicity differences in vaginal colonization with organisms thought to be associated with bacterial vaginosis. Persons of African descent are more likely to have pH>4.5, no lactobacilli, small gram-variable and gram-negative rods and Mobiluncus, compared to Caucasians [45]. In another study, highly significant differences in vaginal colonization were observed, with the highest rates of potentially pathogenic organisms noted in persons of African descent and the lowest in inhabitants of the Asian-Pacific islands [46].

In the present paper, the authors analyzed the possible association between selected demographic and environmental factors which proved to be most important in the pathogenesis of preterm delivery and abnormal microbiological flora of the lower genital tract at early pregnancy. Like other authors, we found that abnormal bacterial flora are present in a large percentage of women in early pregnancy. Our findings revealed that intermediate and abnormal flora were present in 47.9% and in 33.3% respectively of women in early pregnancy. We found that in the group with abnormal flora, the most frequent were G. vaginalis, M. hominis, U. urealyticum, Ch. trachomatis, species which are known to be the main medical cause of chorioamnionitis, PROM, and preterm delivery. On the other hand, we documented that a low educational level, single marital status, first pregnancy, unemployment, and smoking during pregnancy also constitute potential risk factors for abnormal vaginal flora during early pregnancy.

**CONCLUSIONS**

In conclusion, the findings we have reported here may to some extent explain the role and the pathway by which socio-economic and environmental factors may influence the course and outcome of pregnancy. Pregnant women who present risk factors for abnormal cervicovaginal microflora should be included in more comprehensive prenatal surveillance to reduce the related risk of negative pregnancy outcome and make possible the early detection and treatment of this pathology in the course of pregnancy.

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<td>IC Patents</td>
<td>Provides information on patent registration process, patent offices and other legal issues. Provides links to companies that may want to license or purchase a patent.</td>
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